The Effects of Chronic Administration of Excess Iodine on Thyroidal Hormone Synthesis in the Chick*

HOJI SUZUKI, MITSUO SAWADA, KAZUKO TAKAHASHI,
KEISUKE TAKEUCHI AND YOSHIHIKO HORIUCHI

*The Second Department of Internal Medicine,
School of Medicine Hokkaido University, Sapporo*

Synopsis

The response of thyroid weight and iodine metabolism to excess ingestion of iodide was studied in the chick. Treatment of chicks with 1 mg of iodide for 4 weeks induced a marked increase in thyroid weight. The radiochromatography of the ^{131}I labelled thyroid hydrolysate showed that a single injection of carrier iodide resulted in a significant but transient reduction of a relative proportion of organic ^{131}I as well as thyronine ^{131}I and an elevation of the MIT/DIT ratio. After chronic treatment of chicks with excess iodide, the thyronine ^{131}I proportion was still decreased notwithstanding the fact that the organic ^{131}I proportion showed a return to normal and MIT/DIT was rather low. The increased thyroid weight and decreased thyronine proportion returned to normal 9 weeks after termination of the iodide feeding. In the goitrous chicks, the relative proportion of intrathyroidal ^{127}I compounds were virtually identical with that of ^{131}I , although the absolute amount of thyronine ^{127}I was increased depending on the increase in total ^{127}I content. There was no significant difference in both turnover rate of radiothyroxine and plasma thyronine ^{127}I concentration between the goitrous and control chicks. It is suggested that these alterations of iodine metabolism seem to be closely related to the mechanism of goitrogenesis by excess iodide in chicks and may represent an inhibitory effect of iodide on intrathyroidal hormogenesis.

The effect of a large amount of iodide on the thyroid varies with the species (Pitt-Rivers 1960). Wheeler and Hoffman (1948, 1949) first demonstrated goiter in chicks or adult hens by chronic treatment with iodine. On the other hand the rat is resistant to goiter production even by massive doses of iodine (Galton and Pitt-Rivers 1959).

In human subjects, Morgans and Trotter (1953) and others have observed that goiters develop in a small percentage of patients taking iodide compounds for the treatment of asthma or bronchitis. We reported on endemic coast goiters in Hokkaido where inhabitants ingest a

large amount of iodide from kelp (Suzuki *et al.*, 1965). Although the mechanism of goitrogenic effects of iodine has not been completely clarified, the present study was undertaken to study the response of thyroidal hormone synthesis to acute and chronic administration of excess iodide in the chick.

Materials and Methods

Animals

Male or female White-Rock chicks, 9 to 14 weeks of age, weighing 900 to 1,500 g, were fed a commercial diet throughout the experimental period. The iodine content of the diet was approximately 0.2 $\mu\text{g/g}$.

In the experiments in which acute effect of iodide was studied, chicks were given a single injection of ^{131}I (100 μCi of $\text{Na } ^{131}\text{I}$) and carrier iodide (1 mg of

Received for publication June 26, 1969.

* This study was supported in part by Grant No.481/RB from International Atomic Energy Agency.

iodide as KI) intraperitoneally. Control chicks were injected with 10~20 μ Ci of ^{131}I alone. After intervals of 4 to 24 hr., the chicks were killed by decapitation and the thyroids were removed.

In the experiments in which chronic effect of iodide was studied, chicks were given KI in drinking water, so that each animal received about 1.0 mg of iodide per day. After treatment with iodide for 4 weeks, chicks were injected with 1.0 mg iodide and 200 μ Ci ^{131}I . The thyroids were removed 4 and 24 hr. later respectively.

For the study of the aftereffect of a longterm treatment with iodide, the chicks receiving iodide for 4 weeks were killed for thyroid analysis 1, 3 and 9 weeks after discontinuing the iodide feeding. In this experiment, chicks were injected with carrier free ^{131}I and the thyroids were removed 4 hr. later.

The study of the peripheral degradation of radiothyroxine was carried out on control chicks, iodide-treated chick (1 mg per day for 4 weeks), chicks treated with 50 mg of propylthiouracil per day for 4 weeks and chicks treated with 50 μ g of thyroxine per day for 4 weeks.

Column chromatography for analysis of ^{131}I and ^{127}I fractions in the thyroid

The excised thyroids were immediately frozen on dry ice, weighed and homogenized in Krebs-Ringer-phosphate buffer (1.0 cc/100 mg tissue at pH 7.4) containing methylthiouracil (100 μ g/100 mg tissue). The homogenates were hydrolysed with trypsin and pancreatin (7.0 mg/100 mg thyroid tissue respectively) in the presence of toluen for 24 hr. at 37°C. An aliquot of the hydrolysate was adjusted to pH 4.0 and then subjected to column chromatography according to the method of Pileggi *et al.* (1961, 1964).

In order to determine whether or not the column procedure would be applicable to the determination of radioaminoacids in the thyroid, ^{131}I labelled moniodotyrosine (MIT), diiodotyrosine (DIT) and thyroxine (T_4) were added to the thyroid homogenates and then evaluated from the column at pH 3.6, 2.2 and 1.4 respectively. The recovery of the radioiodo-compounds from thyroid homogenates was 98.2% for MIT, 96.9% for DIT and 97.5% for T_4 . Nearly all of Na ^{131}I was retained by the resin in the column.

An aliquot of the effluents from each fraction was subjected to the determination of radioactive and stable iodine. Radioactivity of ^{131}I in each fraction was counted in a well-type scintillation counter and expressed as a relative proportion to the total ^{131}I in the thyroid. A sum of MIT, DIT and thyronine proportions was expressed as an organic fraction. Paralleled analysis of the homogenates was made for

radioiodocompounds by paperchromatography (Roche *et al.*, 1954; Suzuki 1958), and for organic ^{131}I by precipitation with trichloroacetic acid (Pitt-Rivers *et al.*, 1958). The results were virtually identical with that of column chromatography. For the determination of stable iodine, homogenates of the pooled glands from 5 chicks were hydrolyzed and then subjected to chromatography. Analysis of the fractions for iodine content was made following the acid incineration (Gross *et al.*, 1948).

Plasma protein-bound iodine was determined by the method of Grossman and Grossmann (1955). Plasma thyronine iodine was determined by the method of Pileggi (1961, 1964) with a minor modification as follows; 6.0 cc of plasma was subjected to column chromatography using a double volume of reagents. Colorimetry of iodine in the ash was made by the same method of PBI determination as described above (Grossmann and Grossman 1955).

Peripheral degradation of radiothyroxine

50 μ Ci of ^{131}I -labelled L-thyroxine (Abbott Laboratories) was given into a wing vein. After 7, 12, 24, 48 and 72 hr., 1.0 cc of blood samples was collected from the other side of the wing vein in heparinized syringes. Plasma was separated by centrifugation and radioactivity of the plasma ^{131}I was counted as described above. Half-life of thyroxine- ^{131}I ($\text{T}_{1/2}$), thyroxine distribution space (TDS) were calculated according to the method of Ingbar and Freinkel (1955).

Results

Acute effect of iodide

As shown in Figure 1, in the chicks injected with a single dose of ^{131}I and ^{127}I , the relative proportion of organic ^{131}I in the thyroid was significantly decreased 4 hr. after the injection ($p < 0.001$). Thereafter it gradually increased, but 24 hr. later it failed to attain the control level. MIT/DIT ratios in the iodide-treated chicks were significantly higher than control during the first 16 hr. following the injection ($p < 0.001$ respectively). After 24 hr. it returned to the control level. ^{131}I thyronine proportion in the iodide-treated chicks was smaller than the corresponding control value throughout the period ($p < 0.001$ respectively).

Fig. 1. Effect of iodide on the radioiodinated compounds in the thyroid of chicks given carrier free ^{131}I (open circles), ^{131}I with 1.0 mg of carrier iodide (solid circles) and 1.0 mg/day of iodide for 4 weeks (hatched bar).

Bracket indicates the standard deviation of the mean value.

Chronic effect of iodide

As shown in Table 1, the average thyroid weight of the chicks fed iodide was increased to about 1.8 times as much as the control. The difference between the two groups was significant ($p < 0.001$).

Figure 1 also shows the chronic effects of iodide on the relative proportion of ^{131}I constituents in the thyroid. Proportions of organic ^{131}I either 4 or 24 hr. after the injection of ^{131}I were not different from the corresponding control values. MIT/DIT ratio was markedly low in the iodide-fed chicks. The ratio was not only lower than that in the chicks given a single injection of iodide, but also lower than that in the control ($p < 0.001$ respectively). Proportions of ^{131}I thyronine were significantly decreased either 4 or 24 hr. after the injection ($p < 0.001$ respectively).

Total iodine content of the thyroid in the

Table 1. Effect of chronic iodide administration on thyroid weight in chicks

Group	No.	Thyroid weight (mg/kg body weight)
Control	140	$95.2 \pm 32.8^*$
Iodide**	30	176.2 ± 57.4

* Values represent mean $\pm$ standard deviation

** Administered 1.0 mg of iodide/day for 4 weeks

iodide-fed chicks was increased to approximately $820 \mu\text{g/gland}$, whereas it was $120 \mu\text{g}$ in the control. The relative proportions of ^{127}I in the thyroidal iodo compound fractions were almost equal to those of ^{131}I which were obtained 24 hr. after ^{131}I injection (Table 2).

There was no significant difference of ^{127}I thyronine concentration in the plasma between the iodide-fed and control chicks, although in the former, PBI concentration was higher than in the control ($p < 0.001$). The turnover rate of radiothyroxine was significantly increased in the thyroxine-treated chicks ($p < 0.001$) and decreased in the PTU-treated animals ($p < 0.001$). However there was no difference between the iodide-fed and control chicks (Table 3).

Aftereffect of iodide feeding

Figure 2 shows the changes in thyroid/body weight ratios and thyroidal ^{131}I constituents after discontinuing iodide in the goitrous chicks induced by iodide feeding. The weight of thyroid remained greater than that of the control until 3 weeks after discontinuance of iodide. After 9 weeks, however, it was significantly decreased ($p < 0.001$) and returned to an almost normal level.

Radiochromatographic analysis of the thyroids showed no significant change in intra-thyroidal iodo constituents in the chicks 1 week after discontinuance of iodide feeding, as compared to the iodide-fed chicks. However, after 3 weeks the MIT/DIT ratio returned to the control level and ^{131}I thyronine proportion was still decreased. After 9 weeks the chromatogram showed no significant difference from the control.

Table 2. Distribution of stable and radioactive iodide compounds in the chick thyroid

Analysis	Group	No.	Total iodide	Organic iodide	MIT	DIT	Thyronine	MIT/DIT	
¹²⁷ I	Control	(μg/gland)*	120.4	118.4	41.7	39.4	38.3	1.00	
		(%)	—	98.1	33.8	32.9	31.4		
	Iodide**	(μg/gland)*	5	822.0	759.3	104.5	529.5	125.3	0.20
		(%)	—	92.4	12.7	64.4	15.2		
¹³¹ I	Control	(%)	5	—	92.2±4.5	32.8±6.1	31.8±8.3	28.0±4.9	1.05±0.55
	Iodide**	(%)	5	—	91.7±5.1	15.2±7.8	61.4±7.9	15.2±4.8	0.27±0.21

* Obtained from the pooled gland from 5 chicks

** Administered 1.0 mg/day for 4 weeks

*** Analyzed 24 hrs. after injection, the data are expressed as mean and standard deviation

Table 3. Turnover of radiothyroxine and plasma hormone level in chicks fed iodide, propylthiouracil (PTU) and L-thyroxine (T₄)

Group	No.	Half-life of ¹³¹ I-thyroxine (hr.)	PBI ($\mu\text{g}/100\text{ ml}$)	Plasma thyronine ($\mu\text{g}/100\text{ ml}$)
Control	8	9.8 $\pm$ 1.3*	1.07 $\pm$ 0.30	0.65 $\pm$ 0.23
Iodide**	6	9.9 $\pm$ 1.4	1.83 $\pm$ 0.37	0.69 $\pm$ 0.20
PTU***	4	14.5 $\pm$ 5.3	—	—
T ₄ ****	4	7.2 $\pm$ 0.5	—	—

* Values represent mean $\pm$ standard deviation

** Administered 1.0 mg per day for 4 weeks

*** Administered 50 mg per day for 4 weeks

**** Administered 50 μg per day for 4 weeks

Discussion

The results of the present experiments confirm the previous reports that excess iodide has a goitrogenic activity in the chick (Wheeler and Hoffman 1948, 1949).

Radiochromatographic analysis of the chick thyroid for acute effect on iodine showed a decreased proportion of organic ¹³¹I as well as thyronine ¹³¹I and high MIT/DIT ratio, indicating that both the organic binding of iodine and synthesis of thyronine were depressed. In rats fed excess iodide for a long period, relative proportions of thyroidal ¹³¹I constituents were not different from the control (Galton and

Pitt-Rivers 1959; Braverman and Ingbar 1963; Wolff *et al.*, 1949). The chronic iodine effect on the chick seems to be obviously different from the rat. There are a significant decrease in thyronine ¹³¹I proportion and a rather low MIT/DIT ratio in the iodide-fed chicks, suggesting that iodide administered chronically sustains a decrease in the speed of thyronine ¹³¹I formation by the chick thyroid.

Kobayashi and Gorbman (1960) showed no significant changes in the thyroidal radio-compounds in 9 day old chicks treated with 30 μg of iodide per day for 8 days. While, Rosenberg *et al.* (1963a, b, 1964) reported a low MIT/DIT ratio and a decreased thyronine

Fig. 2. Change in thyroid weight and thyroidal radio-iodinated compounds in the chicks during and after treatment with iodide. Thyroids were removed 4 hr. after ^{131}I . In the KI-treated chicks 1.0 mg of carrier iodide was given simultaneously with ^{131}I .

Bracket indicates the standard deviation of the mean value.

^{131}I proportion in both chick and rat, fed on a diet containing 1 to 2 μg of iodine per g. These discrepant results might have arisen from their methodological differences such as dosage of iodine, age of animals or iodine content of diet. However, in our data, goiter is found only in the iodide fed chicks and a low MIT/DIT ratio as well as a decreased thyronine ^{131}I proportion seems to be characteristic to the goitrous chick. Similar findings to the results were previously shown in our report (Suzuki *et al.*, 1965), which indicated a decreased thyronine ^{131}I proportion on endemic coast goiter. In addition, the fact that after discontinuation of iodide treatment the increased ^{131}I thyronine

proportion would appear to support our view.

From the data obtained by estimation of iodide by a direct chemical method, it is evident that a treatment with iodide in the order of magnitude such as used in the present experiment, increases the absolute amount of intrathyroidal thyronine despite a decrease in its relative proportion.

The results of ^{131}I -labelled thyroxine turnover study in the chicks pretreated with PTU or thyroxine, indicate that the half-life of radio-thyroxine ($T_{1/2}$) is a reliable index of the peripheral metabolism of thyroid hormone in chicks. The mean of thyroxine distribution space (TDS) obtained from the iodide-fed and control chicks were 309 ± 96 and 278 ± 139 cc/kg of body weight respectively. Assuming that the degradation of stable thyronine can be expressed as $D = \text{TDS} \times (0.693/T) \times \text{Plasma Thyronine Concentration}$, the mean D for the iodide-fed chick is $3.63 \mu\text{g/day/kg}$ of body weight and 3.08 for the control. These data suggest that in the goitrous chicks the secretion of thyroid hormone from the gland does not change greatly notwithstanding a marked increase in intrathyroidal thyronine. It is generally accepted that thyronine stored in the gland is released by proteolysis of thyroglobulin. In iodine-treated chicks, the proteolytic activity of thyroglobulin was markedly decreased. But, decrease in proteolytic activity was shown also in iodine-treated rats (Takeuchi *et al.*, 1969). Accordingly we believe that the inhibitory effect of chronic iodine treatment on the chick thyroid consists of the combined blocking of coupling of iodotyrosine and depression of proteolysis of thyroglobulin.

It is possible that the above combined block for both synthesis and release of thyronine may initially reduce the amount of hormone secretion and then, depending on compensatory increase in TSH, produce hypertrophy of the thyroid and maintain euthyroidism.

Acknowledgment

The authors wish to thank Professor Keimei

Mashimo for helpful advice in these experiments.

References

- Braverman L. and S. H. Ingbar (1963). *J. Clin. Invest.* **42**, 1216.
- Galton V. A. and R. Pitt-Rivers (1959). *Endocrinology* **64**, 835.
- Gross W. G., L. K. Wood and J. S. McHargue (1948). *Analyt. Chem.* **20**, 900.
- Grossman A. and G. F. Grossman (1955). *J. Clin. Endocrinol.* **15**, 354.
- Ingbar S. H. and N. Freinkel (1955). *J. Clin. Invest.* **34**, 808.
- Kobayashi H. and A. Gorbman (1960). *Endocrinology* **66**, 795.
- Morgans M. E. and W. R. Trotter (1953). *Lancet* **2**, 1335.
- Pileggi V. J., N. D. Lee, O. J. Golub and R. J. Henry (1961). *J. Clin. Endocrinol.* **21**, 1272.
- Pileggi V. J., H. A. Segal and O. J. Golub (1964). *Ibid.* **24**, 273.
- Pitt-Rivers S. R. (1960). *Ann. N. Y. Acad. Sci.* **86**, 362.
- Pitt-Rivers R., V. A. Galton and N. S. Halmi (1958). *Endocrinology* **63**, 699.
- Roche J., S. Lissitzky and R. Michel Method of Biochemical Analysis Vol. 1, Intersci. Publ., New York p. 243 (1954).
- Rosenberg L. L., M. K. Dimick and G. LaRoche (1963a). *Endocrinology* **72**, 749.
- Rosenberg L. L., G. LaRoche and M. K. Dimick (1963b). *Ibid.* **72**, 759.
- Rosenberg L. L., M. Goldman, G. LaRoche and M. K. Dimick (1964). *Ibid.* **74**, 212.
- Suzuki H., T. Higuchi, K. Sawa, S. Ohtaki and Y. Horiuchi (1965). *Acta Endocrinol.* **50**, 161.
- Suzuki M. (1958). *Endocrinol. Japon.* **3**, 291.
- Takeuchi K., H. Suzuki, M. Sawada and Y. Horiuchi (1969). *Endocrinology* in press.
- Wheeler R. S. and E. Hoffman (1948). *Ibid.* **43**, 430.
- Wheeler R. S. and E. Hoffman (1949). *Proc. Soc. Exptl. Biol. Med.* **72**, 250.
- Wolff J., I. L. Chaikoff, R. C. Gokiberg and J. R. Meier (1949). *Endocrinology* **45**, 504.